PRACTICAL

iGEM

PRACTICAL

PETase & Related Analogous Chimera Transfused in Computer & AI Learning

HK_GTC iGEM

Education and Communication
Educational booklet
iGEM 2021

CONTENTS

HOW SERIOUS IS PLASTIC POLLUTION, REALLY?

From common single-use plastic bags to clothing and even fishing nets, plastic can be found in every corner of our planet. It seems that wherever we are, there is always plastic somewhere nearby.

Lightweight and convenient as they are, plastic has taken a crucial role in our society. Our reliance has led to mass production of plastics over the past century, reaching astronomical numbers and showing no sign of slowing down. As helpful as they are in our daily lives, they bring drastic, adverse effects to the environment.

An albatross killed by plastics in Midway Island (1)

Plastics are durable, and doesn't degrade well naturally. Plastic products take years, decades, or often, centuries to degrade. That is what makes it so damaging to the environment.

For example, sea turtles and birds consume plastic products, such as plastic bags and bottle caps, that look similar to their natural prey. This would likely result in their death.

Ingestion of plastic by a sea turtle (2)

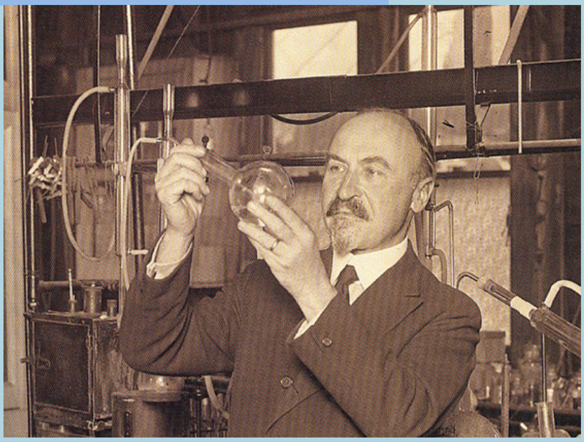
Furthermore, it has been shown that even plankton, the organisms at the bottom of the food chain, consume microplastic when present. Slowly, the plastic travels up through the food chain, until it reaches us, the humans.

Presence of microplastics (3)

Ref:
(1) https://www.onegreenplanet.org/environment/how-chemicals-in-plastic-pollution-harm-you-and-animals/
(2) https://theconversation.com/ghostnets-fish-on-marine-rubbish-threatens-northern-australian-turtles-11585
(3) https://qz.com/448447/plankton-are-eating-plastic-and-that-means-were-all-probably-eating-plastic/

THE TREND OF PLASTIC POLLUTION

Past



Leo Baekeland, the man who invented the first plastic in the world (4)

The first plastic in the world was produced in 1907, marking the beginning of the plastic industry. But people did not realize the large growth of plastic production until the 1950s. However, it was too late even though we realized the issue since it was unstoppable. By the 1990s, plastic waste generation had more than tripled in two decades. In the early 2000s, the output of plastic has risen more in a single decade than in the past 40 years and this show how plastic pollution had been intensified.

Present

Back to now, 300 million tonnes of plastic is produced every year, which is almost equivalent to the weight of all human population in the entire world. With these large numbers, 100% of sea turtles have plastic in their stomachs. 25 trillion pieces of macro & 51 trillion microplastics are littering our oceans.

Photo taken in Lap Sap Wan (5)

Future

Photo taken in beaches of Hong Kong (6)

By 2030, we could be spewing as much as 90 million tonnes of plastic waste into the world's waters like oceans, rivers and lakes. In addition, more than 99% of plastics are produced from chemicals derived from oil, natural gas and coal — all of which are dirty, non-renewable resources. If the current trend continues, by 2050 the plastic industry could account for 20% of the world's total oil consumption. More plastic than fish will be filling up our oceans in 2050.

THE WORLD NEEDS YOU!!!

Ref:

(4) https://chicago.suntimes.com/movies-and-tv/2021/6/27/22551827/all-things-bakelite-review-documentary-leo-hendrik-baekeland-plastic-inventor-movies

(5) https://www.epd.gov.hk/epd/clean_shorelines/activities_2015_3.html

(6) https://www.facebook.com/greenpeace.international/photos/does-this-picture-from-hong-kong-predict-the-future-of-our-beaches-we-hope-not-j/10156029264443300/

10 million tons of plastic are dumped into the ocean every year. This is equivalent to more than a garbage truck load every minute.

As if that's not bad enough, over 50% of all plastic produced every year
(≈ 190 million tons) is for single-use purposes.


A whale found with 29kg of plastics (7)


Thilafushi in the Maldives (8)

Imagine the amount of single-use plastic we throw away — used for just a few moments, but staying on the planet for at least a few lifetimes.

Plastic pollution is an imminent threat. We must act now to save the planet.

Plastic pollution will eventually harm humans and we must act before it gets out of hand.

DIFFERENT TYPES OF PLASTIC

(9)

Polyethylene Terephthalate (PET) typically including drinks bottles and cups.
High-Density Polyethylene (HDPE) including milk jugs, shampoo bottles and grocery bags.
Polyvinyl Chloride (PVC) including rigid plastics like pipes and tubes.
Low-Density Polyethylene (LDPE) such as beer six-pack fasteners and plastic bags.
Polypropylene (PP) used in food containers and some plastic car parts.
Polystyrene (PS) again used to hold food, drinks cups and some plastic utensils.
'**Other**' – A general purpose category for acrylic, nylon and other plastics.
PET, HDPE, PP, PS can be recycled

Ref:
(7) https://www.ptcnews.tv/tag/29-kilograms-of-plastic-in-the-whales-stomach
(8) https://www.worldwildlife.org/blogs/sustainability-works/posts/if-we-want-to-fix-the-plastic-waste-crisis-we-need-to-fix-our-data
(9) https://www.plasticsforchange.org/blog/different-types-of-plastic

GAME CORNER

CAN THESE BE RECYCLED?

Instruction : Base on your knowledge

(10)

Polyester (PET)

Bubble wrap (LDPE)


(11)


(12)

Disposable diapers (PP)

answer : polyester(yes), bubble wrap(no), disposable diapers(yes)

Ref:
(10) https://www.onegreenplanet.org/environment/just-how-bad-is-polyester/
(11) https://sealedair.in/en-gb/product-care/product-care-products/aircap-bubble-wrap
(12) https://www.123rf.com/photo_123107567_group-of-disposable-diapers-arranged-over-a-white-changing-table-hygiene-and-health-care-for-baby.html

HONG KONG GOVERNMENT SCHEMES TO TACKLE THE PLASTIC PROBLEM

01 Plastic Shopping Bag Charging Scheme

Most of us are familiar with the Plastic Shopping Bag Charging Scheme, which has been fully implemented in the retail sector since 1 April, 2015. However, some other schemes and policies are not as widely known by the public.

02 Plastic-Free Takeaway, Use Reusable Tableware Campiagn

For example, the "Plastic-Free Takeaway, Use Reusable Tableware" Campaign aims to encourage the public to go plastic-and-disposable free for takeaway orders to reduce the use of disposable plastic tableware. Nearly 700 eateries participated in the two months that it was carried out, from 10 June to 9 August 2019.

Under the campaign, customers could obtain a sticker for each takeaway made without taking disposable tableware. When 6 stickers have been collected, they could be exchanged for a set of reusable tableware.

03 Reverse Vending Machine Pilot Scheme

You may have also seen these machines on the streets. The Reverse Vending Machine Pilot Scheme started in the first quarter of 2021, and an increasing number of Reverse Vending Machines can now be found in shopping malls or other public places. The machines accept used beverage containers (mostly plastic ones) for a rebate of 0.1 HKD each.

In general, the government follows the "Producer Responsibility Scheme". The polluter-pays principle and the element of eco-responsibility are the key concepts of the PRS. Stakeholders such as manufacturers and consumers should take part in the collection, recycling, treatment and disposal of end-of-life products.

by Hong Kong Government

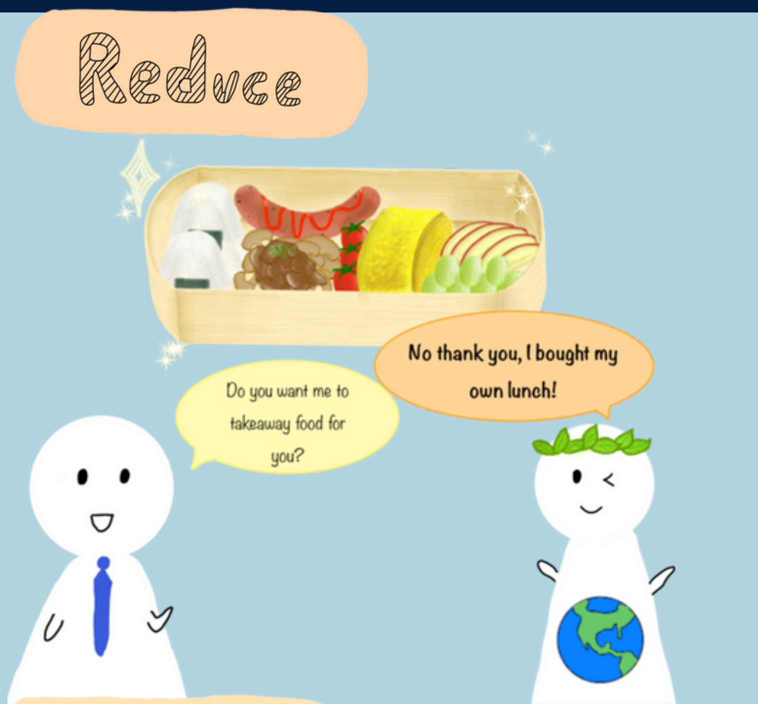
Although the government has done several schemes regarding the issue, we must still take actions by ourselves to solve plastic pollution. What can we do with our limited budget and resources?

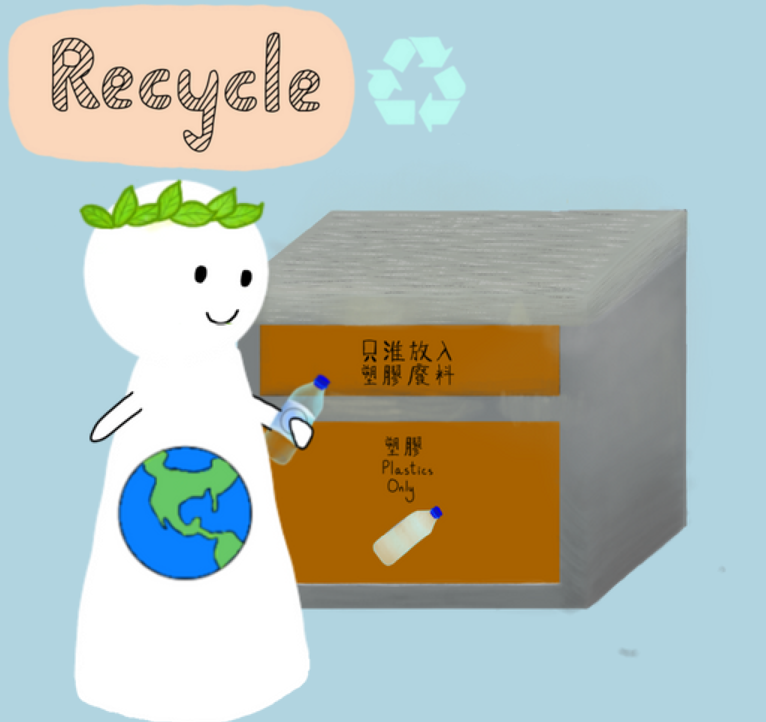
We could adopt the 4R's principle (Refuse, Reduce, Reuse, Recycle) in our lives.

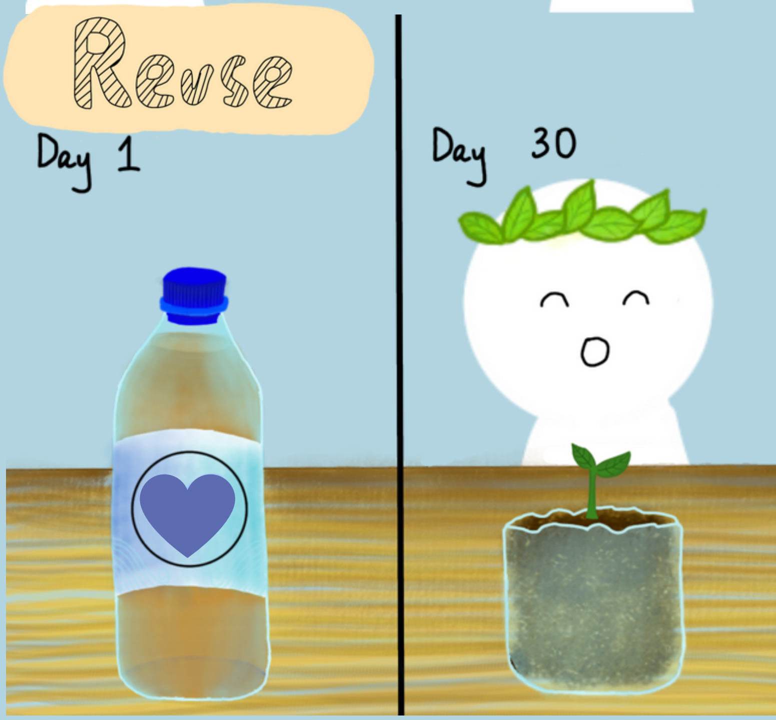
Refuse: Decline unnecessary plastic products
Reduce: Minimize the use of necessary plastic products
Reuse: Repurpose the plastic products before disposing
Recycle: Put the products in recycling bins

By doing so, we could greatly reduce the use of plastic products. With the collaborative effort, every little bit matters.

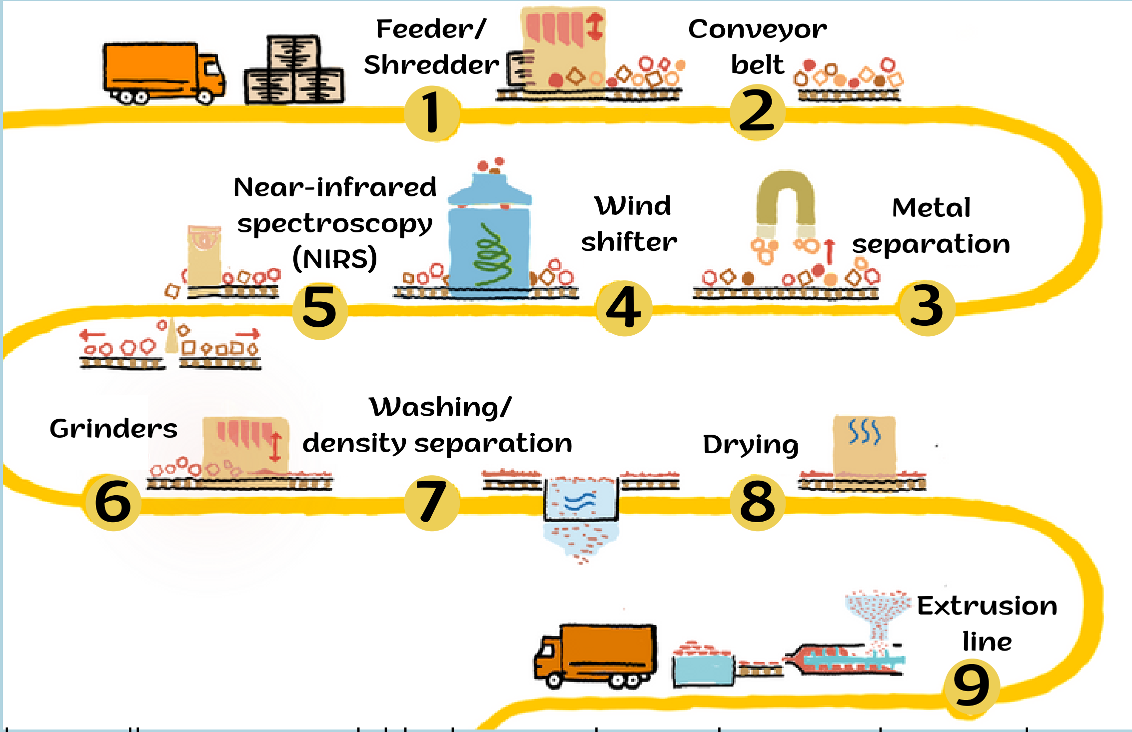
4R'S PRINCIPLE







TRADITIONAL RECYCLING



Admittedly, plastic pollution is unavoidable due to our heavy reliance on plastic products in our everyday lives. We need to find a way to stop plastic from ending up in oceans or our landfills. This is why recycling has become an aspect of increasing interest as of lately.

Traditionally, plastic is collected and sorted in recycling facilities. Then, they are washed to remove impurities, crushed and shredded, and finally melted into tiny pellets, known as **nurdles**. The nurdles can then be made into new products. This process is known as **mechanical recycling**.


(13)

However, this method of recycling brings about a few problems. For example, the transporting of plastic pellets could lead to spills in oceans. In July 2012, After Typhoon Vicente (韋森特) hit Hong Kong, about 150 tons of plastic pellets were scattered in the ocean. Aquatic creatures and seabirds mistake them for fish eggs and therefore consume them.

Besides, plastic wastes are often a contaminated mix of materials. Therefore, the recycled plastic can contain contaminants which decrease the quality of the end products.

Ref:
(13) https://v1.plasticfreeseas.org/

CHEMICAL RECYCLING

Chemical recycling is an approach that we are currently looking into. It involves the breaking down of plastic into its constituting monomers. The monomers can then be reused to produce new plastic, which are better in quality than plastic produced from mechanical recycling.

OUR PROJECT

The aim of our project is to solve the global problem of plastic waste by a protein engineering approach, using a dual enzyme system (with the two enzymes secreted by the plastic-eating bacteria) to chemically degrade polyethylene terephthalate (PET) plastic into constituting monomers that can be further used.

Plastic are polymers, meaning that they are formed by the joining ("condensation") of many units ("monomers"). Usually, they are resistant to natural degradation and are chemically inactive. However, two enzymes, **PETase and MHETase**, secreted by the bacteria ***Ideonella sakaiensis,*** has been found capable of breaking the plastic up into its monomers.

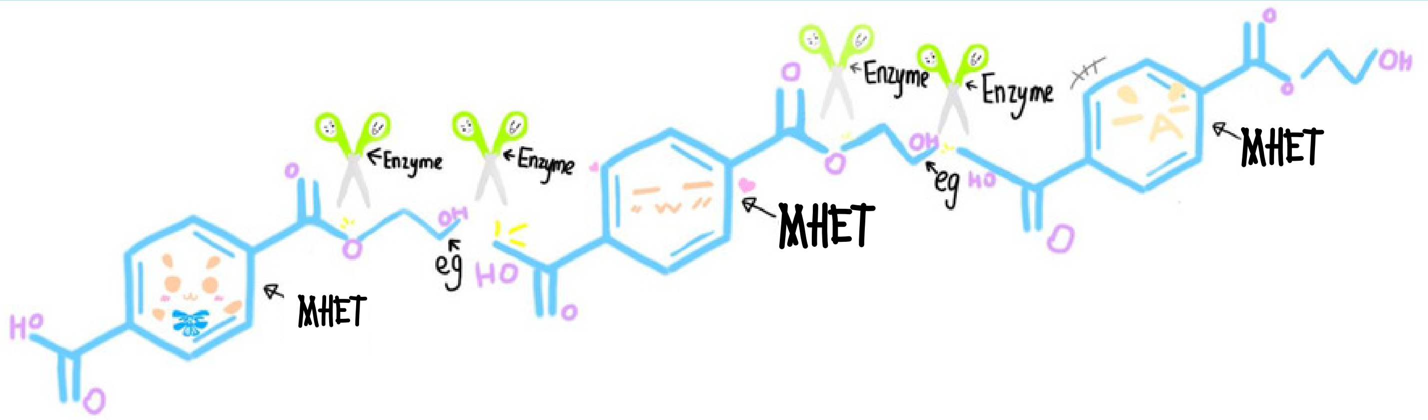
The nature we used is trolling to depolymerize PET plastics with a dual enzyme system. Therefore we hypothesized that the dual enzyme system is also capable to degrading PET plastics in our project.

But first, what are enzymes?

Enzymes are proteins, which is formed by the condensation of different amino acids (氨基酸).

Each protein has a specific 3D structure determined by its amino acid sequence. The shape of enzymes allow them to speed up ("catalyze") certain reactions by providing an alternative pathway of the reaction with a lower activation energy. This is the main function of an enzyme molecule.

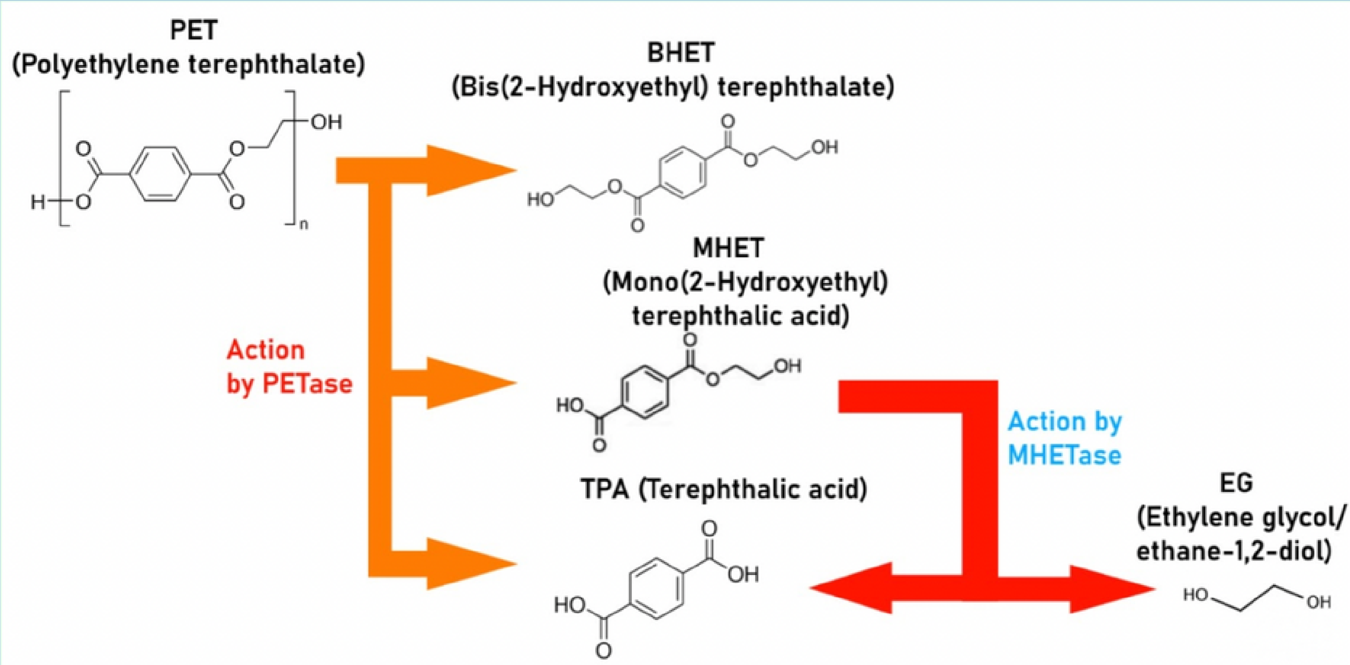
By using the enzymes, we can "cut" the bonds of the polymer and collect the resulting monomers to reconstruct new PET molecules. However, the rate of degradation of PET plastic using PETase is still too low for commercial use.



IN THE PROCESS.........

Therefore, our iGEM team has developed multiple new constructs for the enzymes through genetic engineering as an attempt to improve the hydrolytic activity of the enzymes.

Our newly developed dual-enzyme systems, consisting of either the wild type PETase or our engineered variant S245I and MHETase, are the most successful. The two enzymes are either linked together forming a chimeric protein or mixed together as an enzyme cocktail.

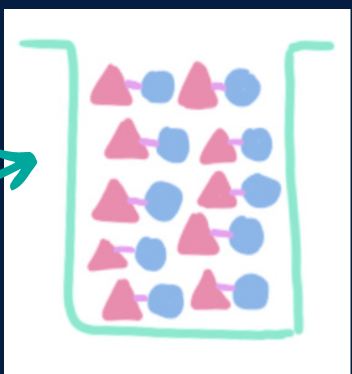


In the process of enzymatic degradation of PET, PET is broken down into BHET, MHET and TPA by PETase. Then, MHET can be further broken down into TPA and EG by MHETase. TPA and EG are the final **monomers** of PET.

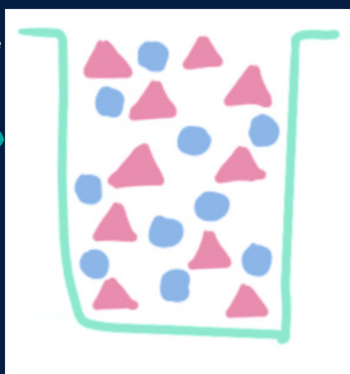
DIFFERENCE BETWEEN CHIMERA AND COCKTAIL

To synergize PET depolymerization process, we develop a dual enzyme system of PETase and MHETase in the form of protein chimeras or enzyme cocktail.

chimera: each PETase protein is linked with the MHETase protein by a chain of 12 amino acids.



cocktail: a mixture of the two enzymes in a solution

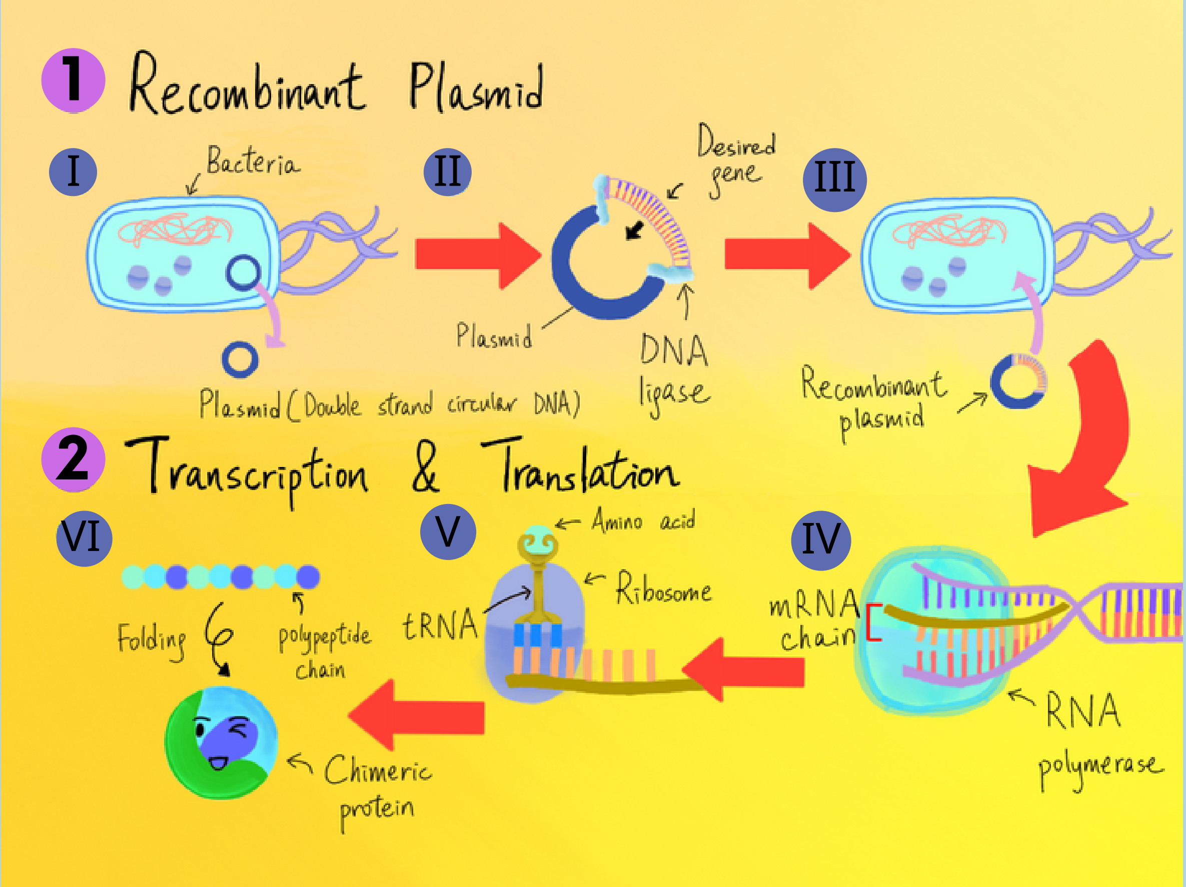


PETase/ Mutated PETase S245I

MHETase

The dual enzyme system can degrade PET into its monomers more efficiently so that we can have a **better efficiency** in recycling!

GENETIC ENGINEERING

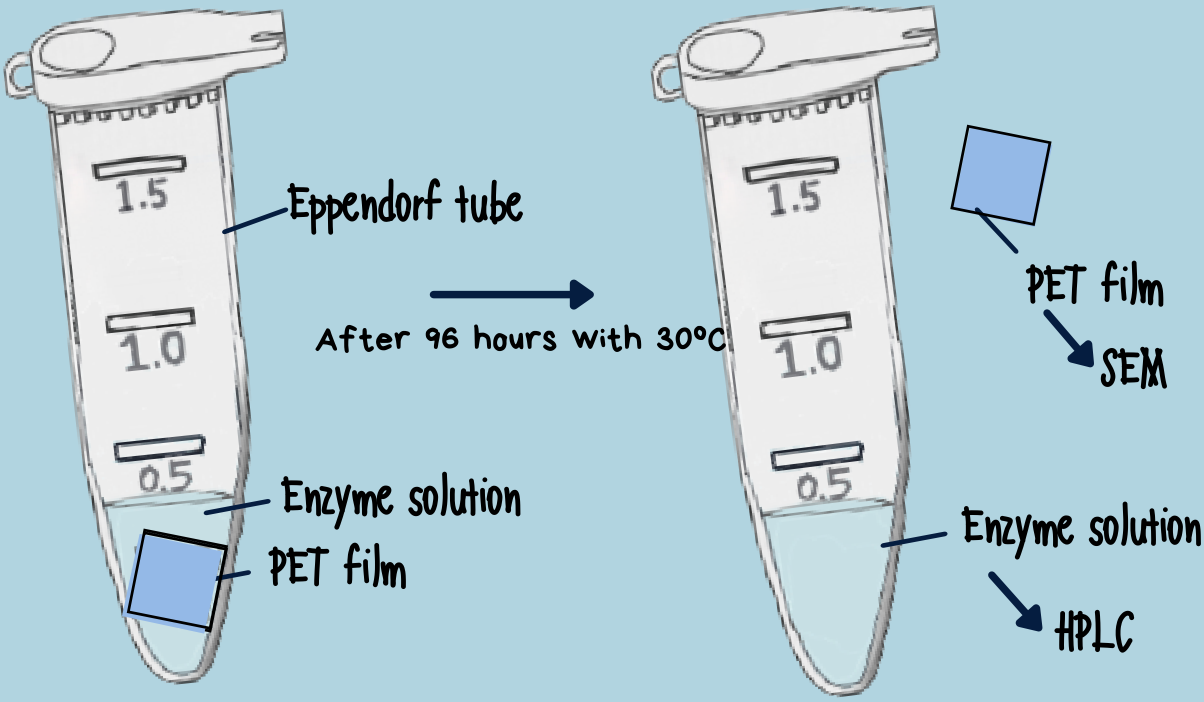


As we all know, PETase and MHETase are useful in recycling. Going back to the production of these "magical" enzymes, they are the outcome of the process of genetic engineering.

Gene is a section of DNA. Different genes have different functions due to their various base sequences. If we want to incorporate a new function into an organism, we can insert the desired gene which carries the information into this organism as the genetic code is universal amongst almost all organisms. Genetic engineering gives new features to organisms, which is why it is utilized in agricultural activities to boost the nutrition values of crops and may even serve as a possible way to tackle plastic pollution!

(I) **Plasmid**, which is double-stranded DNA in circular form, can be extracted from the bacterial cell.
(II) Restriction enzymes cut DNA at specific base sequence.
(III) Same restriction enzyme cuts both the plasmid and the desired gene, which are then joined by DNA ligase. The new plasmid is known as a **recombinant plasmid.**
(IV) The recombinant plasmid is then introduced into the bacterial cells for replication.
Transcription
(V) **RNA polymerase** binds to the location of DNA where transcription should begin. According to the complementary base pairing, a **messenger RNA** strand is synthesized.
Translation
(VI) The messenger RNA binds onto ribosomes. One end of the **transfer RNA** carries a specific amino acid and the other end matches with the three bases in mRNA. Peptide bonds form between two adjacent amino acids and a peptide chain is formed.
(VII) When the entire mRNA is translated, the peptide chain folds onto itself to form a **protein**, which, in this case, is our mutated PETase and chimeric proteins.

PET FILM DIGESTION EXPERIMENT

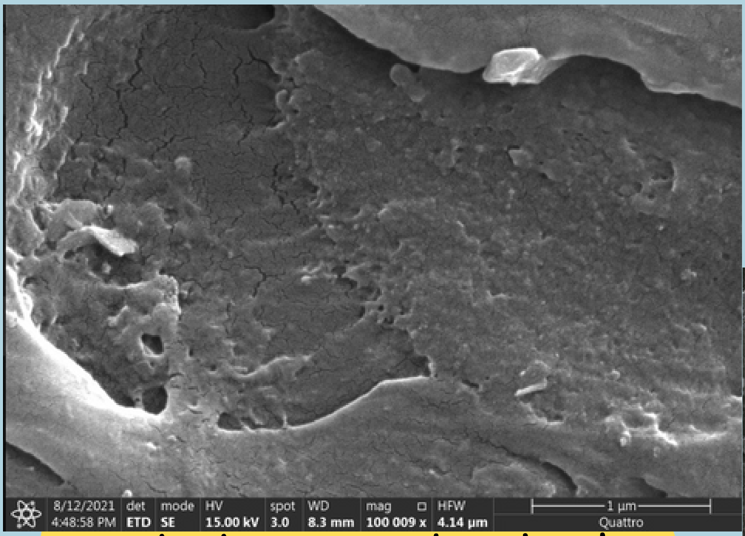


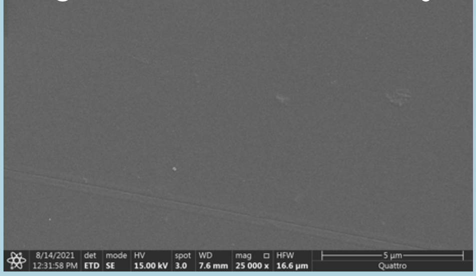
EXPERIMENT RESULTS

SEM photos

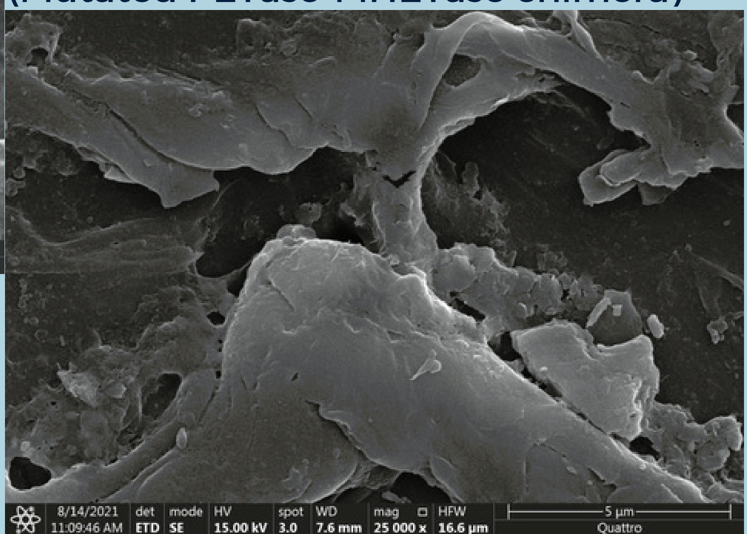
We used a **scanning electron microscope** (SEM) to see the erosion of the PET film surface, after digestion with PETase and MHETase. By comparing the SEM photos of different batches of enzyme combinations, we can see the differences in their ability to digest PET plastic.

S245I+MHETase
(Mutated PETase+MHETase enzyme cocktail)




Negative control (Buffer only)

S245I-MHETase
(Mutated PETase-MHETase chimera)

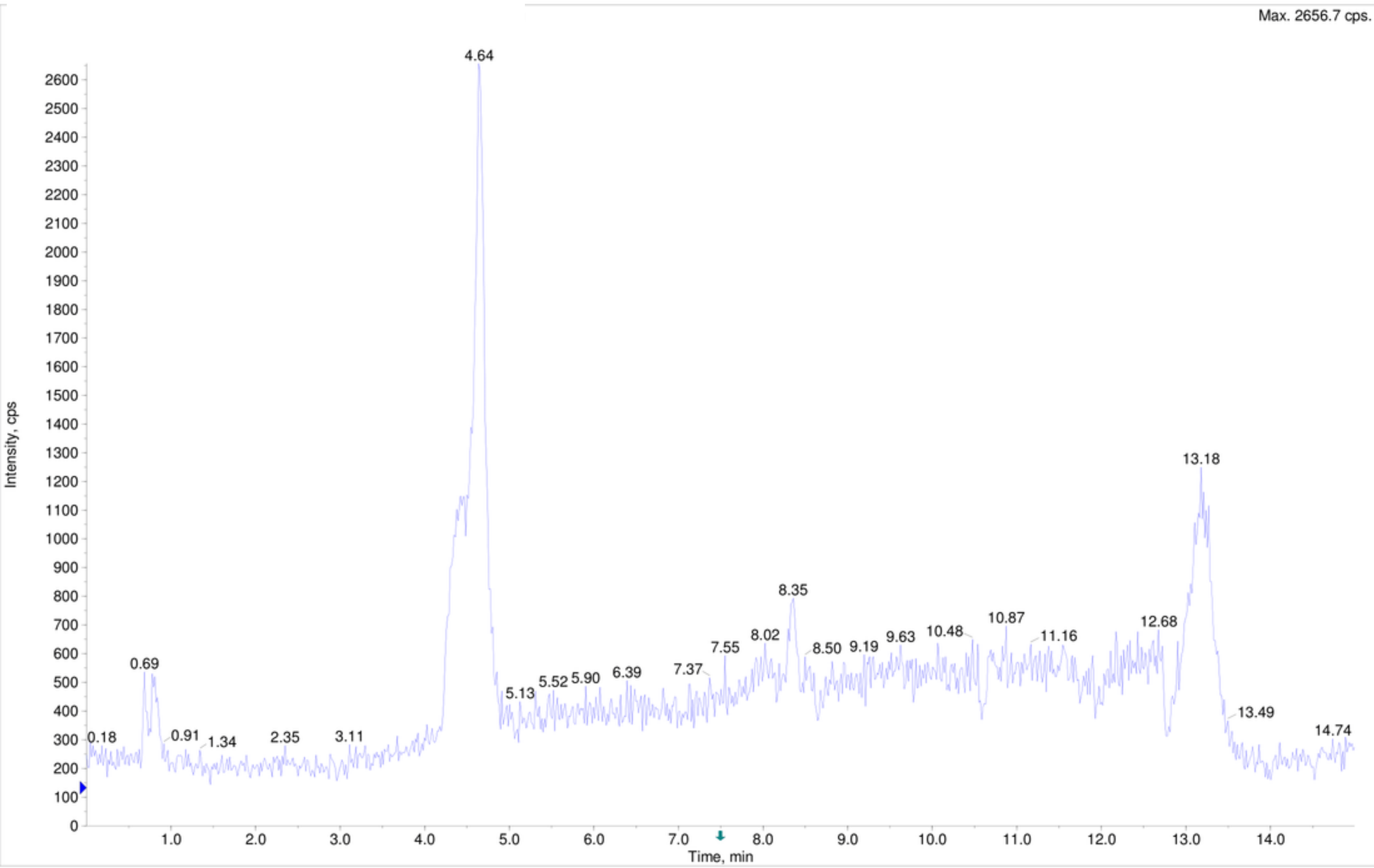


Both chimera and cocktail: shows pitting on PET film
negative control: no sign of digestion

HPLC data

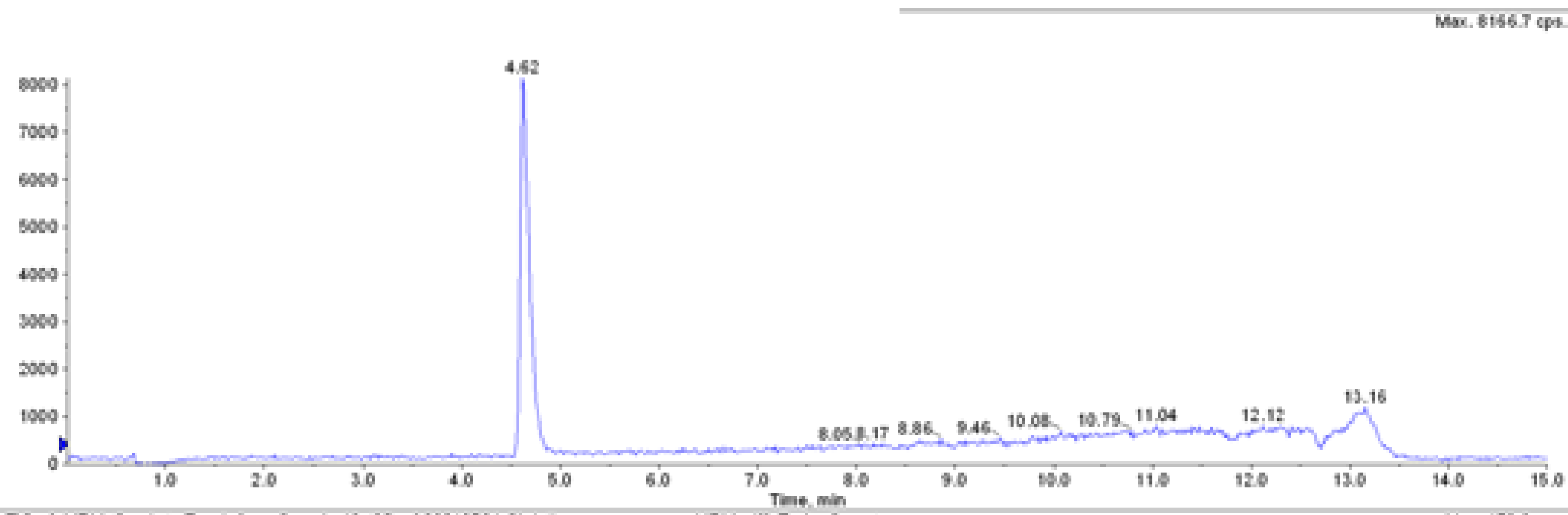
HPLC (high-performance liquid chromatography) was used to test the TPA level in the solution containing PET film after being digested by different constructs of enzymes.

TPA Standard



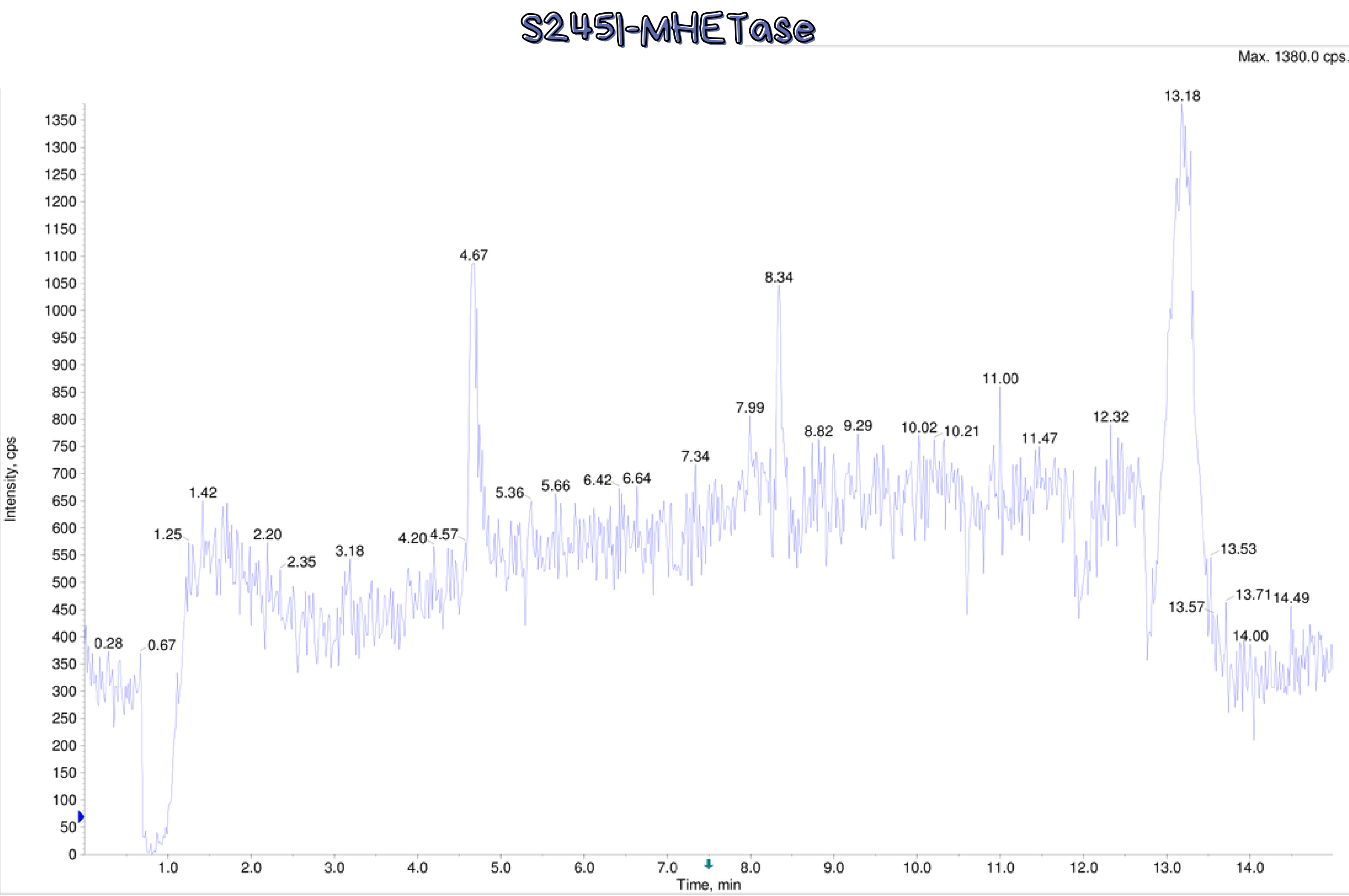
4.64 min is the standard retention time for TPA.

S245I+MHETase



This data is obtained by analyzing the supernatant of PET film solution after digestion by S245I+MHETase. The intensity of TPA is around 8156.7 cps, which shows S245I+MHETase enzyme cocktail can digest PET into TPA.

HPLC data



This data is obtained by analyzing the supernatant of PET film solution after digestion by S245I-MHETase. The intensity of TPA is around 1150 cps, which shows S245I-MHETase protein chimera can digest PET into TPA

DRONE & AI

Aim

Drone & AI technology was applied to train and develop a deep learning PET bottle detection model for mapping plastic pollution on beaches. Our work is divided into three parts: plastic photography, plastic tagging and the model training programme.

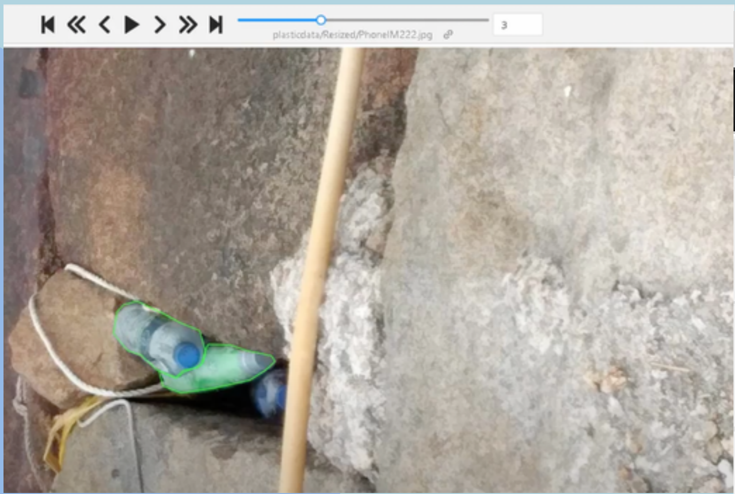
Plastic Photography

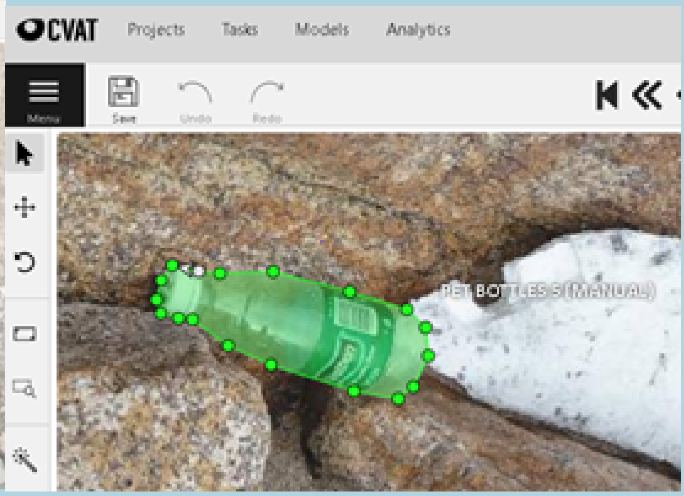
Our team has visited Lantau Island, Cheung Chau and Lei Yu Mun to conduct our plastic photography so that we can get a grasp of the plastic problem in general in Hong Kong. We have brought our drone, (DJI Phantom 4 Pro) along with us to take pictures of around the coastlines.

We have taken 718 images with the drone and our phones.

Plastic Tagging

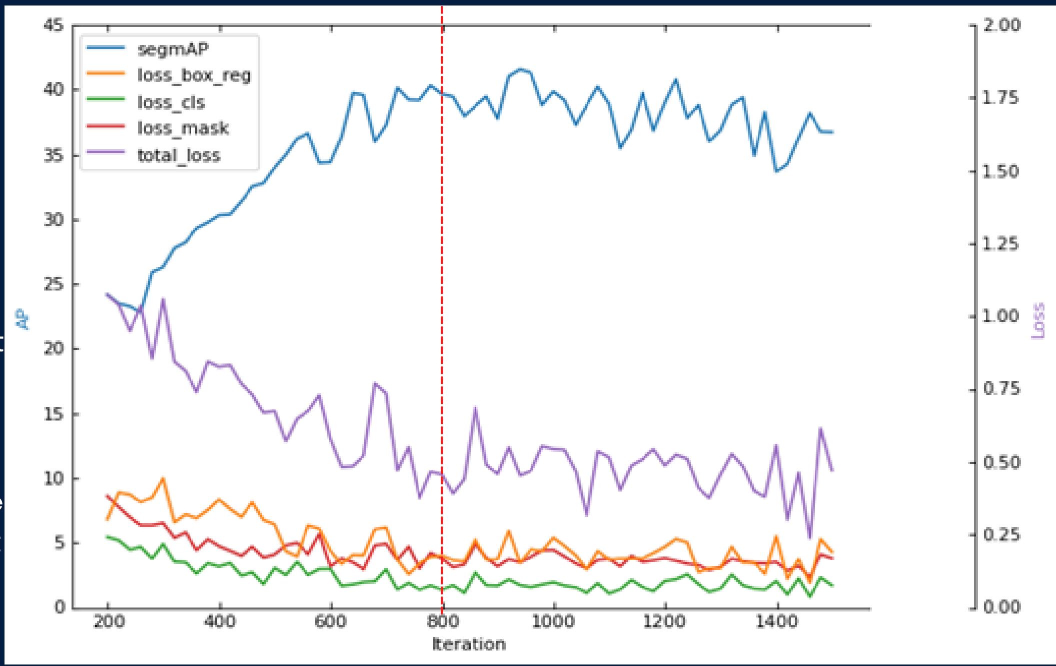
We have invited student volunteers from our school to help us tag the plastics in the images using the platform CVAT. We have filmed a video teaching them how to tag the plastic step by step. After that, we utilised Mask RCNN (object detection algorithm) to detect the plastics and train our AI model.
Our AI model achieved a stable accuracy after 500 iterations which shows that our model is successful!





TRIAL MODEL TRAINING RESULTS

After training the model we collect the result of both AP and total error from every 20 iterations and plotted them in a graph. Both of the values have achieved stability at around 800 iterations, so the amount of training should be enough for seventy-three images. we use ResNet 50 as our feature extracting backbone network, for simplicity it's just a convolutional network with 50 layers. And



2 images per step for mini-batch stochastic gradient descent. And with a total of 1 thousand and 5 hundred iterations. We use seventy-three images for training and twenty-three images for validation, in a ratio of 7:3. And data augmentation is also used, including random flipping and random brightness to increase training efficiency.

Our Model

AP	AP50	AP75	APs	APm	APl
36.7393	53.5804	43.5817	0	45.5128	24.0369

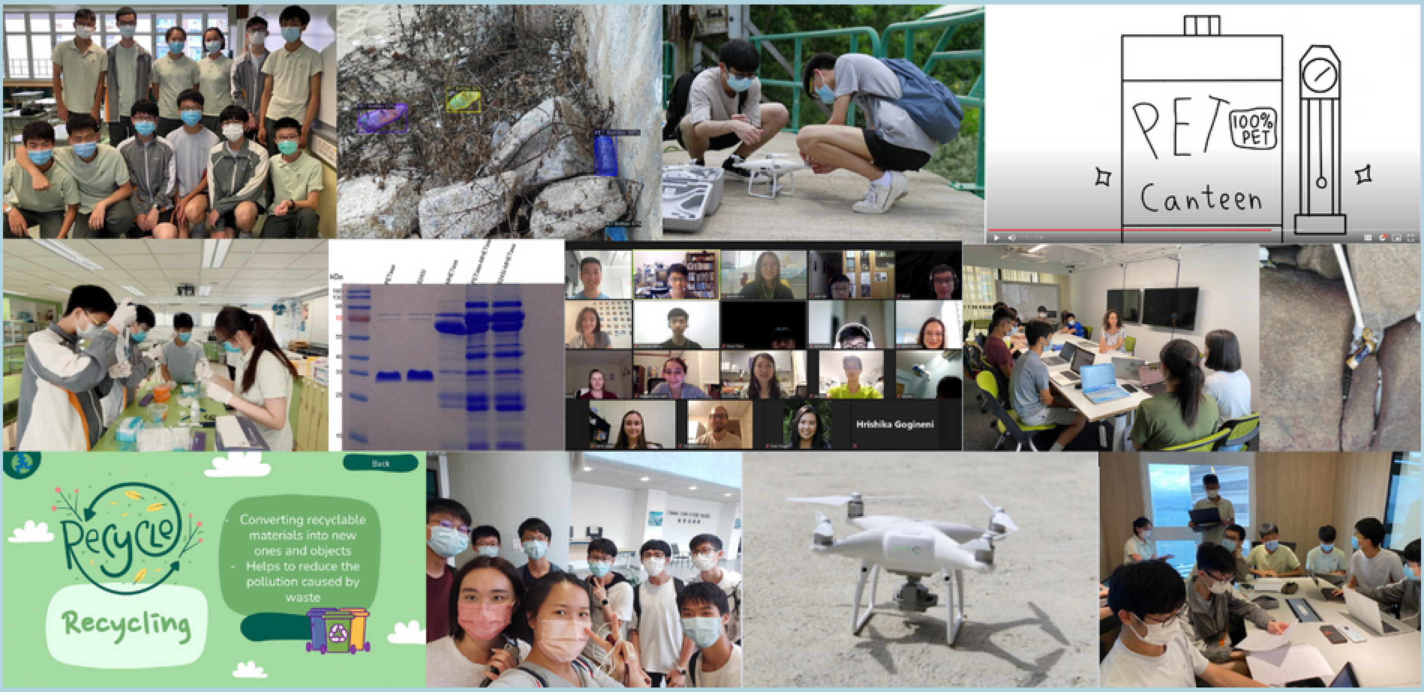
From Mask-RCNN Paper

	backbone	AP	AP_{50}	AP_{75}	AP_S	AP_M	AP_L
MNC [10]	ResNet-101-C4	24.6	44.3	24.8	4.7	25.9	43.6
FCIS [26] +OHEM	ResNet-101-C5-dilated	29.2	49.5	-	7.1	31.3	50.0
FCIS+++ [26] +OHEM	ResNet-101-C5-dilated	33.6	54.5	-	-	-	-
Mask R-CNN	ResNet-101-C4	33.1	54.9	34.8	12.1	35.6	51.1
Mask R-CNN	ResNet-101-FPN	35.7	58.0	37.8	15.5	38.1	52.4
Mask R-CNN	ResNeXt-101-FPN	**37.1**	**60.0**	**39.4**	**16.9**	**39.9**	**53.5**

Compared to the Mask-RCNN Paper Benchmark, where they are using a ResNet 100 for the backbone network, to detect various classes of objects. We got a final result of thirty-six point seven AP, which is similar to the results from the papers - thirty-seven point one.

By comparing the plastic tagging results by the volunteers and the results of our model. The model results are very close to the ground truth annotations, therefore our model training results are successful.

THANK YOU!



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